3085-Pos Board B240
Action of Daptomycin on Membranes
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Daptomycin is a clinically important 13 amino acid lipopeptide antibiotic. Its N-terminus is acylated with n-decanol and its C-terminal 10 amino acids form a ring. It is structurally and size-wise different from other well-known membrane-active antimicrobials. Daptomycin is known to disrupt the cytoplasmic membrane function of Gram-positive bacteria by causing leakage of potassium (and potentially other) ions, leading to the loss of membrane potential and cell death. The critical factor affecting the function of daptomycin is its interaction with negatively charged lipids such as PG in a calcium (Ca^{++}) dependent manner. Based on previous research on cell membranes, daptomycin has been assumed to insert and aggregate in the membrane, and then to alter the membrane curvature. However these details have not been demonstrated by biophysical studies. In our aspirated GUV experiments, we found that with a DOPG-containing GUV and a sufficient concentration of Ca^{++}, daptomycin can extract lipid molecules, and form lipid-peptide aggregations. The lipid-peptide aggregates did not occur if cardiolipin replaced PG, or if other divalent ions, such as Mg^{++}, Ba^{++} replaced Ca^{++}. Similarly, daptomycin did not bind to a GUV when cardiolipin substituted for DOPG or in the absence of Ca^{++}. Daptomycin with Ca^{++} did bind to a pure DOPC GUV, but had no other effects. Furthermore, with the presence of daptomycin, DOPG and Ca^{++}, we found Ca^{++} permeates into the GUV, while a content dye, Texas red dextran, did not leak out. This result suggests that daptomycin and Ca^{++} do not form pores on the membrane of DOPG-contained GUV, but cause leakage of ions. Finally, daptomycin with DOPG and Ca^{++} produces a negative exciton CD couplet centered at the 225 nm absorption peak of Trptophan1 and Kynurenine13, whereas in all other conditions, the exciton CD couplet is positive.

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Amino Acid Sequence and Membrane Binding for a Series of Closely related Amphipathic Peptides
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We have investigated the dependence of peptide activity on the amino acid sequence for a series of synthetic peptides derived from δ-lysin. δ-Lysin is a 26 amino acid, N-terminally formylated, hemolytic peptide that forms an amphipathic α-helix bound at membrane-water interfaces. A shortened peptide, lysette, was derived from δ-lysin by deletion of the 4 N-terminal amino acid residues. Five variants of lysette were synthesized by altering the amino acid sequence under the constraint that the overall hydrophobic moment be essentially the same for all peptides. Peptide-lipid equilibrium dissociation constants and helicities of peptides bound to zwitterionic lipid vesicles were determined by stopped-flow fluorescence and circular dichroism. We then compared the thermodynamics of peptide binding calculated using the Wimley-White hydrophobicity scale with the experimentally determined free energy of binding. We found a systematic deviation of the experimentally determined dissociation constant and that predicted by the Wimley-White scale. Molecular dynamics simulations suggest two factors that account for the very favorable experimental binding free energy. (1), in all δ-lysin-derived peptides simulated, the initial bilayer contact is made with the polar peptide face, allowing charged residues to establish strong interactions with the bilayer headgroup region early on. (2), if the two aspartate residues contained in the lysette sequences are located at the C-terminus, they remain exposed to water and are, thus, effectively removed from the bilayer headgroup region.

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Synergistic Cell Permeabilization by External Electrical Pulses and New Anticancer Peptides Designed on the Basis of the Cry11Bb Protoxin
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Electrical aspects of the membrane permeabilization by various polycationic peptides were studied. The peptides were designed on the basis of synthetic 16-mer and 14-mer fragments of the Cry11Bb protoxin by their conjugation to the cell penetrating hepta-arginine vector trough two glycine residues. Some of these peptides demonstrated selective killing of human leukemia Jurkat cells but not of the normal wild type CHO cells. In this respect, the designed peptides were more active than the known anticancer peptide R7-KLA. The peptides permeabilized the energized mitochondria as well as the red blood cells with relatively high plasma membrane potential generated in the presence of valinomycin. The efficiency of the peptides was remarkably higher in the lower ionic strength media. The capability of the plasma membrane permeabilization by the designed peptides was strongly potentiated by the external high voltage electrical pulses applied to the cell suspension. Similar effect was observed using the planar lipid membrane, demonstrating that the formation of peptide pores in the lipid bilayer is highly increased by an increase in the applied transmembrane potential (minus at the trans-side). The obtained results open the perspective of the local destruction of solid tumors using the combined ''peptide-electrical pulses'' synergistic treatment. Colciencias (Colombia) research grants #111840820380 and #111852128625.

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Membrane Permeability of Peptides and Drugs
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Amphipathic peptides bind to the outer leaflet of membrane bilayers. Do they translocate across the membrane? The answers are directly related to their functions in the case of cell-penetrating peptides and drugs, or to their mechanisms in the case of AMPs. To our knowledge, few permeability experiments have been performed so far, probably because of the difficulty of detecting the peptides and also because a meaningful test must be conducted at biologically relevant concentrations which are usually low. In the case of pore forming peptides, the test must be performed without pore formation. Here we demonstrate a membrane permeation experiment for a peptide drug NYAD-1 (an HIV-1 inhibitor). There are several factors that make this experiment possible. We used a FITC labeled peptide called NYAD-2 for the peptide detection. We discovered that it is possible to produce GUVs in pH 9, although not in pH 7. We found that the intensity of FITC in pH 9 is 1.7 times higher than in pH 7. By using an aspirated GUV, we measured the relative binding coefficients of NYAD-2 to the GUV in pH 7 and pH 9, and found that the former is 2 to 3 times higher than the latter. With all these provisions, we performed the permeability test by transferring an aspirated GUV produced in pH 9 solution containing Texas red dextran (TRD) MW 625 to a solution of pH 7 containing 2 micro-Mole NYAD-2. We found the concentration of NYAD-2 inside the GUV increased with time while the membrane remained intact and there was no leakage of TRD. We are able to extend this method to test the membrane permeability of other peptides and drugs such as Melittin.

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Effects of Sequence Length and Composition on Antimicrobial Peptide Action
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Antimicrobial peptides are short, cationic, amphiphilic naturally occurring sequences that have selective antimicrobial properties. The proposed mechanism of action for these peptides is through interaction with and disruption of the bacterial membrane. We have investigated the length and amino acid composition dependence of the antimicrobial peptides ponericin L1 and C18G on antimicrobial activity and membrane binding ability. Truncation of the peptide sequence at different points through synthesis allowed for investigation of length, overall hydrophobicity, and net charge on function. Circular dichroism and fluorescence spectroscopy were used to determine the binding affinity and structure of the peptide in the presence of lipid vesicles. All of the L1 derived peptides exhibited the ability to form alpha helices and bind to the lipid membranes to different degrees. The data suggests that the modified peptide (L1A) and the truncated peptides (L1A-13T, L1A-16T, and L1A-21T) work by forming an alpha helix to permeabilize the bacterial membrane and cause bacteriolysis. Alternatively, the C18G derived truncates exhibited a length threshold in their ability to bind membranes with high affinity and form helical structures. Ongoing experiments are probing membrane topography.

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Interaction of Magainin 2 with Gangliosides as a Target for Human Cell Binding
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It is important to understand how AMPs interact with human cells for development of antimicrobial agents of systemic use or anticancer drugs. However, litte is known about the mechanisms by which AMPs bind to them and exert cytotoxicity. Negatively charged gangliosides on cell surface are a potential target for cell binding. [1] In this study, we investigated the interaction of MG2 (F5W-magainin 2) with gangliosides in detail. MG2 was colocalized with gangliosides on HeLa cells, indicating that gangliosides act as a receptor for MG2. Physicochemical studies using liposomes revealed that MG2 interacts with monosialoganglioside GM1 differently from the typical bacterial anionic lipid phosphatidylglycerol (PG). MG2 bound to GM1 more strongly than to PG, and the bindig isotherm for GM1 could be analyzed by the Langmuir equation assuming the charge neutralization. This makes a contrast to the binding of AMPs